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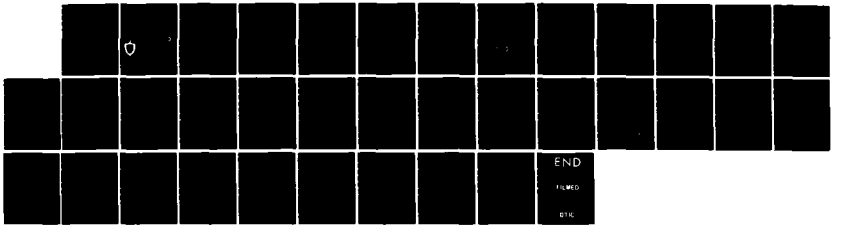

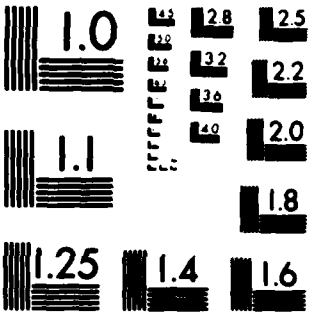

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NOTES ON USE OF THE SINGLE SOLUTION ASCORBIC ACID METHOD FOR PHOSPHATE DETERMINATIONS: AN AMENDED METHOD

BY

THEODORE G. TOWNS, Ph.D.

DECEMBER 1984

TECHNICAL REPORT

ENGINEERING DIRECTORATE
ROCK ISLAND ARSENAL
ROCK ISLAND, ILLINOIS 61299-5000

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5. Calibration Curves
6. EPA Nutrient Quality Control Samples
7. Modified Ascorbic Acid Method
8. Molybdophosphoric Acid

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The project objectives were to define the sources of potential inaccuracy and imprecision in ortho-phosphate analyses and to find a practical and reliable solution to quantitation ~~on the interval~~ 1 ppm $\leq [PO_4^{3-}] \leq$ 6 ppm with the use of 1.0 cm polystyrene cuvettes. The objectives were successfully completed. A modification to the current "standard method", involves heating samples, after addition of the color developer reagent, at 75 ~~deg~~ °C for ca. 20 minutes. The modification yields quantitative results with high precision, high accuracy and birtually no constraint on the timing of absorbance measurements.

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FOREWORD

This report was prepared by Dr. Theodore G. Towns, Howard University, Washington, D.C. The work was accomplished during the Summer of 1984 while Dr. Towns was employed under the Historically Black College Program for professors.

The project was carried out in the Analytical Chemistry Laboratory, Engineering Directorate, Rock Island Arsenal. The author would like to thank the Branch Chief, Mr. Samuel L. Williams, Team Leader, Mr. William F. Garland, and Environmental Coordinator, Dr. William Shore, for their encouragement and support throughout this project. Drs. Shore, Richard W. Perry, and John Moriarty are especially appreciated for the numerous enlightening and helpful discussions on this topic. Finally the author would like to thank Mr. J. W. McGarvey, Chief, Materials Science Division, for his insight in approving the pursuit of the data reported here.

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1.0 INTRODUCTION

1.1 Survey of Phosphate Analysis.

The text, "Standard Methods ..." [1], describes several methods for the determination of phosphorus (as phosphate) in aqueous samples. The determination is based on the development of a colored molybdophosphoric acid complex which has well defined absorption in the visible or near-infrared. The intensity of the color developed, i.e., the magnitude of the optical absorption, is taken as directly proportional to the concentration of phosphate present.

The total phosphate present in a water sample is represented as the sum of so-called organic and inorganic phosphate. When the analyst is interested in the total phosphate content, the method prescribes a preliminary digestion process for the purpose of converting the organic phosphate fraction to inorganic (ortho-) phosphate; one then selects one of three colorimetric schemes for quantitation. The specific colorimetric scheme is adopted in consideration of the expected concentration range and desired sensitivity. The digestion processes (perchloric acid, sulfuric and nitric acid, or persulfate) are of different rigor and complexity of execution, and because of the complexity, reference 1 recommends that the persulfate digestion be compared with either of the others and adopted if recovery is comparable or acceptable. For a complete description of the types of phosphate, and a detailed presentation of the analytical construct for their separation and determination, the reader is referred to reference 1.

The three colorimetric methods are, in order of decreasing sensitivity using 1.0 cm optical pathlength as reference: single-solution ascorbic acid; stannous chloride (Tin (II) chloride, $SnCl_2$); and vanadomolybdophosphoric acid. The ascorbic acid scheme happens to be the most precise and accurate, the most convenient and the most recently adopted by reference 1. (Each method derives its name from the specific reducing agent responsible for the characteristic color developed by the molybdophosphoric acid complex; i.e., ascorbic acid, stannous chloride, or vanadate ion.)

1.2 Method Characteristics Pertinent to Adoption.

Ascorbic Acid Method. This method calls for the addition of a freshly prepared (0.1 M) ascorbic-acid-based color developer (CD) to a phosphate-containing sample and then measurement of absorbance at λ = 880 nm after not less than ten minutes and not longer than thirty minutes. This constraint on the time of measurement, t_m, contributes to the method's relative simplicity. The significance and implication of t_m is a major topic of section 2.0, Discussion, in this technical report.

Stannous Chloride Method. Again, after addition of a freshly prepared $SnCl_2$ (0.11 M) based CD, absorbance at λ = 690 nm is measured. The constraint on t here is the most stringent of the three methods: not less than 10 minutes and not greater than 12 minutes. The color develops, then fades. In addition, there is a temperature contraint here: samples and reference solutions must be between 20 deg C and 30 deg C, and they should all be within two deg C of each other. This temperature constraint need not be a problem practically, but is nonetheless an additional concern.

Vanadate Method. After addition of the CD, absorbance is measured at λ = 400 nm, 420 nm or 470 nm depending on the concentration range of interest; t_m must be greater than five minutes; there is no specified temperature constraint.

1.3 Problem Statement.

Previous analyses for phosphate, using the single-solution ascorbic acid method, have been occasioned with erratic and often unacceptable recovery of phosphate certified US EPA quality control samples particularly in the region 1.0 ± 0.1 ppm and above. The situation is depicted in Figure 1 (cf., Tables I and II). The features of note are the excellent linear correlation between standards and recovery of Quality Controls (QC) and Spikes (e.g., SPK-A) on $0.1 \leq [PO_4^{3-}] \leq 0.9$ ppm , the marked change in sensitivity (slope) at ca. 1 ppm to yet another (less) linear segment of the calibration "curve," and the equally remarkable scatter about any part of the curve of spikes in the neighborhood of 1 ppm. This latter behavior is also observed for numerous daily calibration checks. While one might easily accept such variance as expected near regions of high "curvature" in a calibration curve, a disconcerting note is that the difference between SPK-DD and SPK-D is the value of t and that SPK-B has nearly the same t as SPK-D yet is well removed from any correlation. While such results can often be attributed to either inadequate reference material, inappropriate calibration samples (i.e., an ill-defined analytical response function) inexperienced personnel, or the "breakdown of Beer's Law," the nature of the prescribed procedure [1] and the concentration range of interest indicated that a closer, more detailed inspection of the methodology would be useful. Further, insufficient appreciation for "timing," as can easily happen with many colorimetric analyses, may lead to such inaccuracy and imprecision.

The present study, while engaging the definition of potential problem areas directly affecting the accuracy and precision of phosphate determinations, has been conducted from a perspective based in the following questions.

(a) How sensitive are results to the volumetric apparatus used for dispensing sample and standard aliquots (i.e., is the graduated cylinder sufficient, or must one use volumetric pipets and flasks throughout).

Beer's Law Plot for Standards and Quality Control Samples

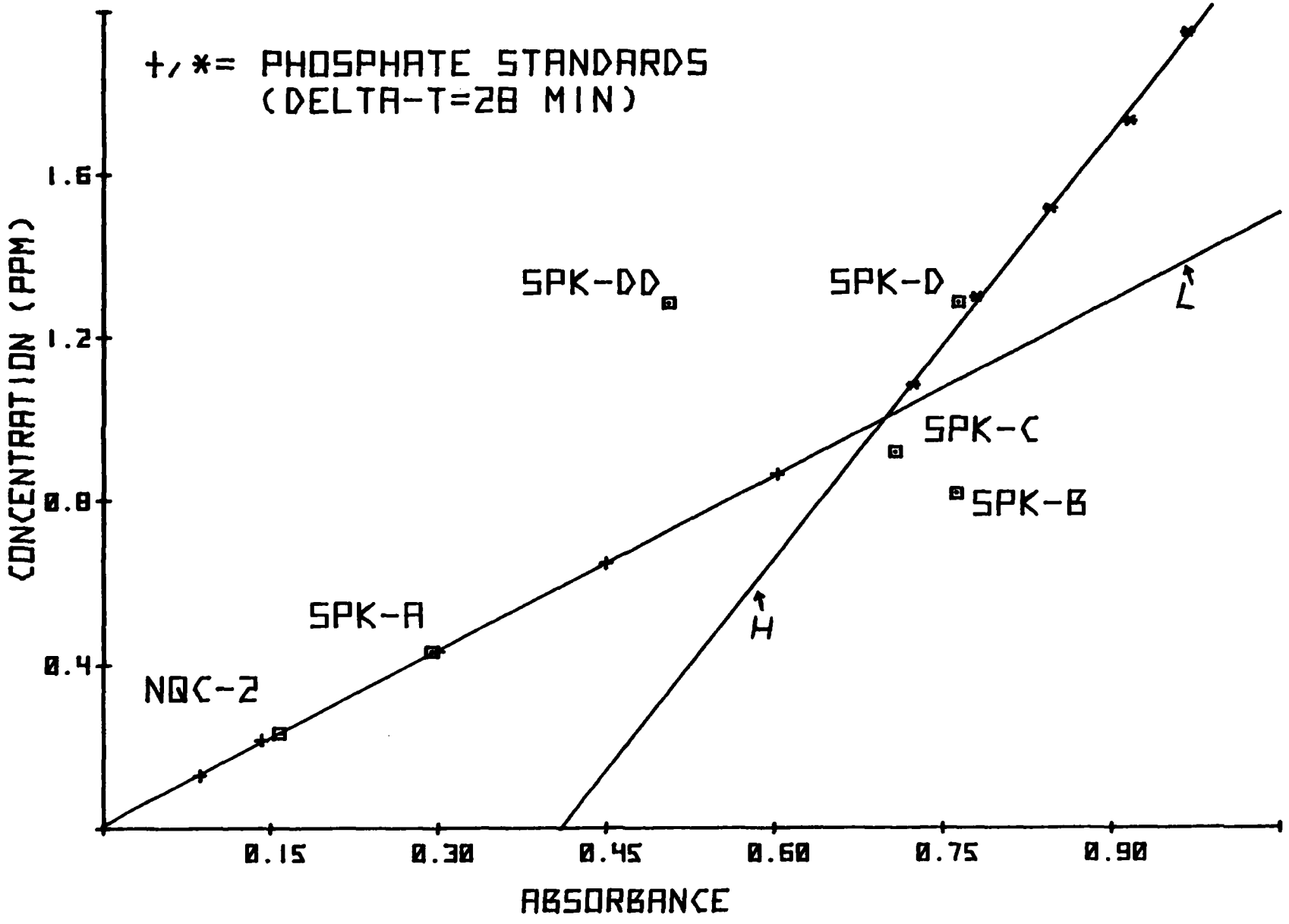


FIGURE 1

TABLE I (1)

Precision and Accuracy of Published Methodology

	t_m (minutes)							
Replicate Number	25' NQC-2	31' SPIKE-A	25' SPIKE-B	17' SPIKE-C	28' SPIKE-D	18' SPIKE-DD	18' NCQ-1	18' SPIKE-M
1	0.159	0.306	0.752	0.705	0.772	0.506	0.023	0.073
2	0.158	0.303	0.757	0.700	0.770	0.511	0.023	0.069
3	0.161	0.305	0.763	0.715	0.771	0.510	0.023	0.069
4	0.160	0.287	0.762	0.712	0.723 (2)	0.502	0.022	0.070
5	0.159	0.288	0.770	0.709	0.773	0.507	0.022	0.068
6	0.158	0.287	0.770	---	0.760	0.507	0.021	0.077
7	0.159	0.286	0.761	---	0.782	0.505	0.021	0.067
$\bar{A}$	0.159	0.294	0.762	0.708	0.764	0.507	0.022	0.070
S_A	0.001	0.009	0.006	0.0006	0.018	0.003	0.0008	0.002
RSD, %	0.6	3	0.8	0.8	2.3	0.6	3.6	2.8
$[PO_4^{3-}]^{ppm}_{obs}$	0.232	0.425	1.09	1.01	1.22	~0.03 (3) ~0.07	32.5 ppb	96.2 ppb
$[PO_4^{3-}]^{ppm}_{True}$	0.233	0.430	0.814	0.916	1.28	1.28	40 ppb	99.4 ppb
Recovery, %	99+	98+	134	110	95	---	77% (4)	99+

(1) See appendix 5.2 for details on the (true concentration) of spikes.
(2) Rejection of this point, by whatever criteria, gives an observed concentration = 1.25 ppm (97% Recovery).
(3) Upper number derived from calibration curve on $[PO_4^{3-}] > 0.8$ ppm. Lower number derived from calibration curve on $[PO_4^{3-}] < 0.8$ ppm c.f., Figure 1.
(4) US EPA acceptable (2σ) recovery interval for this QC is ± 50%, i.e, 20-60 ppb.

TABLE II

Linear Regression Data for Figure 1

LINE L

CORRELATION COEFF= 0.99991
A= 1.415
B= 0.009
Y=AX+B

X	Y	Y,PRED	RESIDUAL	%RES
0.088	0.129	0.133	-0.004	-3.268
0.142	0.216	0.210	0.006	2.974
0.300	0.431	0.433	-0.002	-0.534
0.450	0.646	0.646	0.000	0.070
0.603	0.862	0.862	-0.00	-0.003

LINE H

CORRELATION COEFF= 0.99868
A= 3.450
B= -1.414
Y=AX+B

X	Y	Y,PRED	RESIDUAL	%RES
0.725	1.078	1.087	-0.009	-0.847
0.780	1.293	1.277	0.016	1.256
0.846	1.509	1.505	0.004	0.288
0.917	1.724	1.750	-0.026	-1.464
0.968	1.940	1.926	0.014	0.750

(b) Are the specified values of sample (50 mL) and color developer (8 mL) aliquots critical, or simply their ratio, or neither.

(c) Must one use glass spectrophotometer cuvettes, or are the disposable (polystyrene or acrylic) variety sufficient.

(d) What is the sensitivity and range available through the routine use of 1.0 cm pathlength cuvettes.

(e) How sensitive are results to the time of measurement, t_m.

(f) With exclusive use of 1.0 cm pathlength, what is the shape of the calibration curve between the posted detection limit (10 ppb) and, say, two or three ppm.

Since the purpose of any of the digestion procedures is to convert organically bound and any other "polyphosphates" to ortho-phosphate, the focus of this report concerns primarily this latter type. That is, scant attention has been given the "total phosphate" analysis, since an initial trial for total phosphate gave excellent results.

1.4 Objectives.

The objectives of this report are:

(a) To provide quantitative answers to all of the questions posed above.

(b) To provide documentation of the accuracy, precision, range and sensitivity available under normal procedures using the single-solution ascorbic acid spectrophotometric method.

(c) To introduce an efficacious modification to the "standard method."

2.0 DISCUSSION

2.1 Experimental Details.

In general, the procedure outlined in reference 1 was followed with particular attention to the precautions given. All glassware in any way involved in the total procedure, after scrupulous cleaning with a non-phosphate containing detergent and rinsing with tap then deionized water, was rinsed three times (or soaked for not less than ca. 10 minutes) with hot (ca. 60 deg C) dilute HCl [2]. Specific glassware was allocated and sequestered for phosphate analyses only. Vessels not in use were stored filled with deionized H_2O. The mixed reagent, referred to herein as the (color) developer or CD, was usually prepared immediately before its use, but in no case was developer used for which the elapsed time from its preparation was greater than four hours. Ascorbic acid solution (0.01 M) was not kept longer than four days. Reagent stocks were kept under refrigeration (4 deg C) when not in use. For digested samples, standard solutions were also taken through the digestion process. The digestion procedure calls for a final pH adjustment with stock NaOH to a "faint pink;" since consultation with other intra- and extramural chemists indicated this final adjustment to be crucial, as the end point was approached, NaOH was added dropwise until a "barely discernable" pink was achieved.

All analytical activity (volumetric preparations, dispensing sample and standards aliquots, spectrophotometric measurements, etc) was carried out at an ambient laboratory temperature near 23 deg C. Previously refrigerated reagents were allowed to reach this room temperature before use. The absorbance data for calibration curves and for quality control samples (QC's) and QC + spike (SPK-letter) were obtained using disposable, polystyrene, 1.0 cm pathlength cuvettes with covers. The aliquot for both samples and standards was 20.0 mL and for CD was 3.20 mL (with the exceptions noted in Appendix 5.2); this is the same proportion of sample to CD specified in the method. The constituents of the CD were always mixed in the proportions of concentrations and manner specified by the method, while the specific volumes used were scaled up or down according to the required total volume of CD (allowance made for rinsing the dispensing pipet). The developer was prepared in a ground-glass stoppered graduated cylinder, which facilitated thorough mixing after each addition. Also for dispensing convenience, CD components were kept in 300 mL polyethylene squeeze bottles.

All aliquots were measured in a 100 mL graduated cylinder. Spikes (one of the standard solutions) were always added from a class A volumetric pipet. For example, 20.0 mL aliquots of EPA Nutrient Quality Control - # (e.g., NQC-2) were placed in fourteen 125 mL Erlenmeyer flasks and to seven of the flasks an aliquot of spike was added. At the designated time the same volume of CD, dispensed from a graduated 5 mL Mohr-type pipet, was added to each flask which was swirled to insure timely mixing.

Absorbance measurements were made at λ = 880 nm on a Bausch and Lomb model 710, single beam (digital readout) spectrophotometer (200 nm to 1000 nm; 0.000 A to 2.000A) equipped with a three position cell holder. Solutions with out-of-range absorbance were diluted (1:1) with distilled water and remeasured in the same cuvette after rinsing. The linearity of the instrument response at 880 nm checked to specification against a set of optical filters (Chemetrics Corp, model SS-10) designed for the purpose. The reagent blank (CD diluted with distilled H_2O to its concentration in each sample) remained in position 1 so that it could be checked between samples; the reagent blank was used to set the instrument's 0.000 A. Before use, the absorbance versus air of each disposable polystyrene cuvette was measured; this procedure was maintained until a sufficient sampling ($n > 120$) was obtained to characterize the optical consistency of the cells (A (cell) = 0.090 ± 0.004). In all cases, an attempt was made to choose cells randomly from their packaging case, their optical surfaces were visually inspected for scratches, smudges, films or other blemishes, and a cell was rejected if such defects were noted.

2.2 Computational Details.

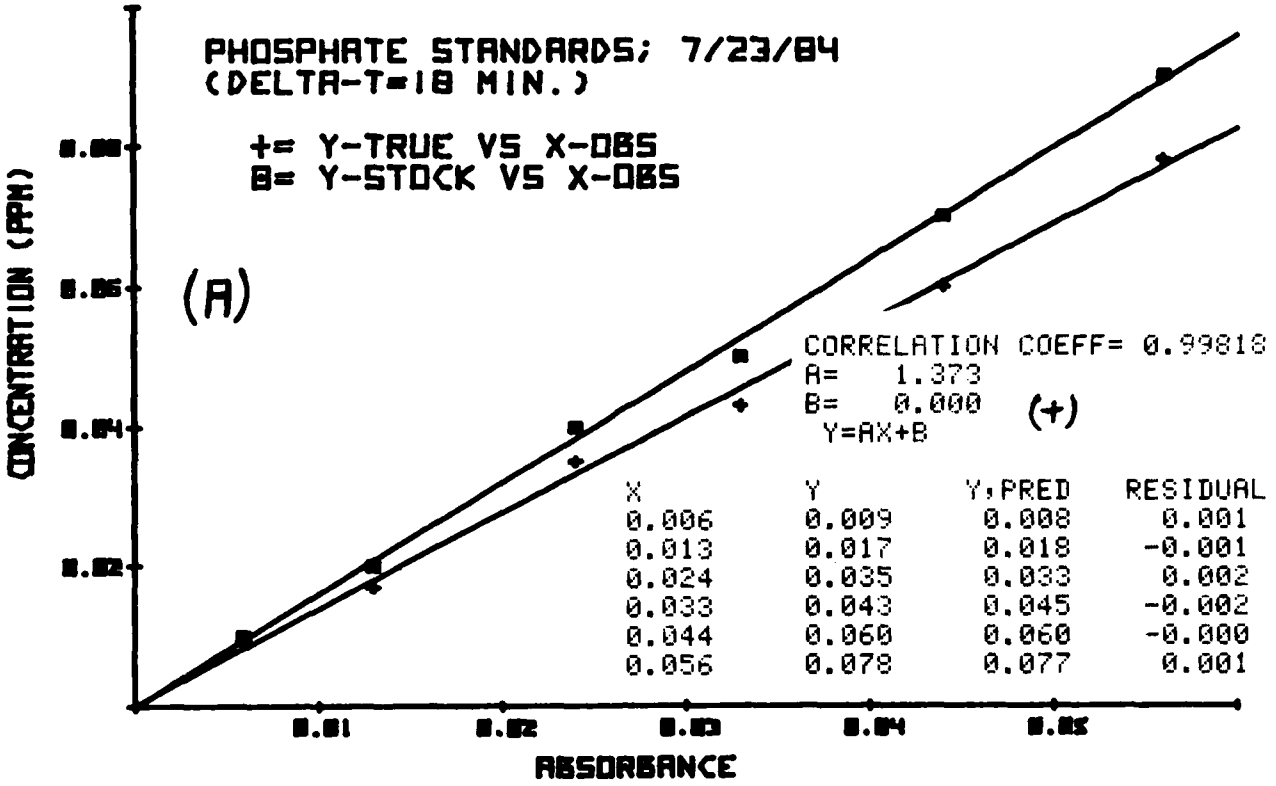
The prepared concentration of each of the stock standard solutions used to construct the calibration curve is not the actual or true concentration of phosphate responsible for the observed absorbance of each such solution, because the dilution of the standard aliquot by addition of the developer occurs. The dilution factor will depend on the proportions of sample and developer aliquots used. While the precision of the analytical results may not be affected by oversight of this fact, significant loss in accuracy of the quantitation can easily result thereby. Figure 2 addresses this point, where the two lines presented are stock (prepared) concentration and true concentration versus observed absorbance, respectively. The difference between the two lines increases with concentration; this relationship between the two plots exists regardless of the interval to be calibrated. Thus, an otherwise acceptable variance in the empirical measurement may be easily misconstrued as inaccuracy or poor or unacceptable precision. By the same token, unacceptable quality may be so disguised.

For the above reasons, as well as simplicity, all calibration curves presented here have been constructed as true concentration versus observed Absorbance; similarly, the QC recovery data has been so plotted in these figures.

2.3 Implications of the Methodology.

The results presented in the next section demonstrate that the single-solution ascorbic acid spectrophotometric determination of phosphorus as phosphate, as prescribed in reference 1:

Beer's Law Plots for True and Stock Concentration Values



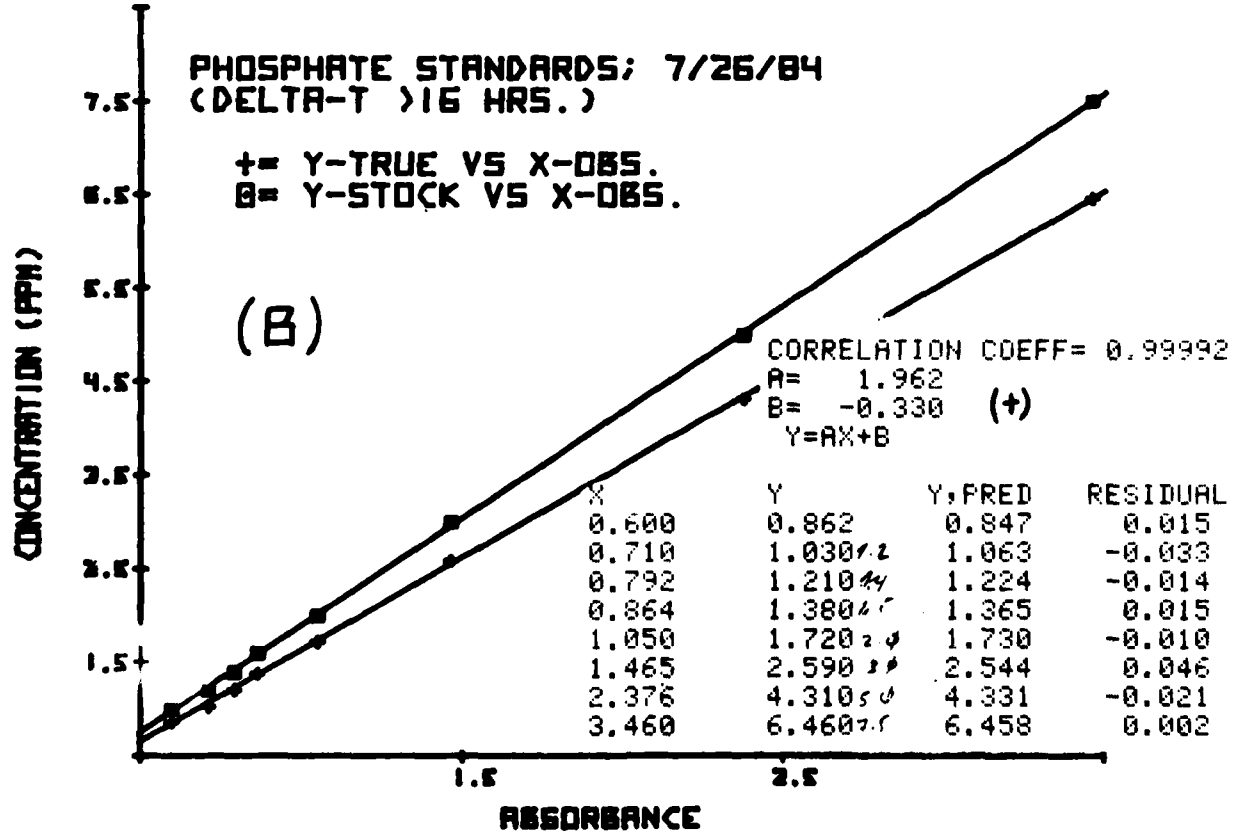


FIGURE 2

(a) is a method capable of high precision and accuracy over the range from at least 7.5 ppm to its posted detection (quantitation) limit of 20 ppb, with the exclusive use of 1.0 cm pathlength, disposable cuvettes; (b) is subject to significant (predictable, nonetheless) variability in analytical achievement caused by the brevity of its procedural exposition; and (c) is procedurally based on contingent assumptions because of, for example, a lack of sufficient background information about the physical chemistry of the analytical system.

Thus, before presenting the data and quantitative results, a stepwise examination of the implications of the "prescription" for phosphate determination is worth while. Present discussion, in turn, assumes that all phosphorus is present as dissolved ortho-phosphate and that pH has been adjusted with sulfuric acid or sodium hydroxide to the interval 8.2 to 9 pH units (phenolphthalein indicator); i.e., that the sample is ready for addition of the color developer. (For the remainder of this section only, "sample" is used variously to denote sample unknown or standard solution; the context should make clear any specific meaning.)

> To a 50 mL sample aliquot add 8 mL of the combined reagent (CD).

Two reasonable inferences are made here: (1) the appropriate proportion of CD to sample is 8:50 (at the trace determination level); and (2) the proportionality bears only one significant figure, i.e, the method is relatively insensitive to the exact volume of CD dispensed.

The former inference is perhaps obvious. But regardless of the validity of the latter, which is easily inferred from the experimental details of the original journal article [2], section 2.2 above, makes clear the importance of the "exact" CD volume, while convenience and simplicity in data reduction would dictate also that it be constant throughout any given set of samples. (An initiate might also easily construe 50 mL to be the only appropriate aliquot.) A more definitive assessment of either the volume or proportion of CD must await studies designed for such purpose.

> After at least 10 minutes but no longer than 30 minutes, measure the sample Absorbance at λ = 880 nm.

Adequate appreciation of this step is at the heart of the method's consistency in both precision and accuracy. The implications here are that color intensity, i.e., maximum absorbance proportional to the total concentration of ortho-phosphate present, is fully developed anytime after 10 minutes; and further, that the constancy of the proportionality deteriorates after 30 minutes. A major purpose of this report is to demonstrate that the latter implication is decidedly false on $[PO_4^{3-}] > 1$ ppm and therefore that the former (symbolically, $(dA/dt) = 0$) is subject to the constraint, $[PO_4^{3-}] \leq 1$ ppm.

Cell pathlengths appropriate to approximate ranges are:

l (cm)	$[PO_4^{3-}]$ (ppm)
5.0	0.01 to 0.25
1.0	0.15 to 1.3
0.5	0.3 to 2

The implications here are sweeping and potentially the cause of considerable consternation to the analyst. They are:

(a) That the Lambert (pathlength) aspect of the Beer-Lambert Law (cf., Appendix 5.1) is indeed linear with and indentically zero intercept, thus allowing simple proportionality between, for example, sample data collected with a 0.5 cm cell and a calibration curve established with a 1.0 cm cell. There are no known deviations from Lambert's Law for a homogeneous medium, particularly as a first order interaction with the low powers typical in traditional dispersive spectrophotometry. However, if $(dA/dt) \neq 0$, then even when t_m is the same for both the 0.5-cm-path-data and the 1.0-cm-path-calibration-curve, the absorbance measurements not only do not correspond to the total amount of phosphate present but also do not correlate with equal fractions of the total phosphate present. Murphy and Riley [2] examined with three inch (7.62 cm) pathlength cells the range 0.02 ppm $\leq [PO_4^{3-}] \leq$ 0.25 ppm, on which $(dA/dt) = 0$.

(b) That preparedness for the range of phosphate concentrations routinely encountered in environmental monitoring requires maintaining a stock of varied pathlength sample cells, else the analyst is faced with a potentially interminable process of dilution or concentration of samples during quantitative (as opposed to limiting value) analysis.

2.4 Data and Results.

The accuracy and precision available from the method is exemplified by the data in Table I; remember that each column of data was generated under (what this author considers to be) an impractical stringent adherence to the timing of absorbance measurements. The t_m values given at each column head are accurate to $\pm$ 10 seconds (which is the approximate delivery time for the volume of CD added) and is applicable to each row entry. The data of Table I is also represented as Y-true vs A-observed in Figure 1. Note that the apparent acceptability of the analytical response to spikes C and D is caused entirely by circumstantially fortuitous values of their respective concentrations, and to the proximity of their t_m values to those of the standard solutions.

The linear regression data for the two sets of standards shown in Figure 1 are given in Table II. The increased scatter (smaller correlation coefficient) for line II data might be easily dismissed as trivial and attributed to either an artifact of lower sensitivity or normal variability in technique. In the present case, however, this difference was taken as not only significant but implicative of stability

problems regarding the color development. And indeed, as shown in Figure 3 which for emphasis spans a wider range of concentrations, the color is not "fully" developed for $[PO_4^{3-}] \geq 1.21$ ppm during the currently prescribed time interval for measuring the absorbance. Further, the fraction of development sustained by each standard decreases with increasing concentration. These two factors either individually or together account for the widely held impressions that for $[PO_4^{3-}] > 1$ ppm the calibration curve is non-linear or Beer's Law has "broken down." A final note on Figure 3 in comparison with line H of Figure 1 can now be made. When a linear correlation between standards on $[PO_4^{3-}] > 0.86$ ppm; t_m 16 hours is attempted, the degree of correlation, and more importantly the slope, is dramatically contingent on both t_m and the number and spacing of calibration points used. Therefore, the reliability of such measurements is indeed only fortuitous and dependent on an unrealistic synchrony between t_m (calibrations) and t_m(samples).

Since 16 hours at room temperature (23 deg C) represents little if any improvement over prior sample dilution, the data of Figure 3 suggested that increasing the temperature of the developing solution would speed the color development to completion. Therefore, seven 20 mL aliquots each -- of eight standards on $0.2 \leq [PO_4^{3-}] \leq 6.46$ ppm plus one lower concentration QC plus two previously troublesome spikes plus one blank were prepared, mixed with a constant volume (3.20 mL) of CD, heated in a 75 deg C water bath for 20 minutes, brought to near room temperature under the cold water tap, and then measured for absorbance at 880 nm. During heating, each Erlenmeyer flask was securely covered with Parafilm to minimize concentration changes by evaporation; after cooling, each flask was inverted with the covered opening pressed against the palm to recover condensate from the Parafilm and the neck of the flask. All absorbance measurements were made versus an unheated blank; the heated blank in each set showed zero absorbance indicating the the photochemical system had not been altered. The statistical analysis of the spikes in Figure 4 is given in Table III. During the procedure that generated the Figure 4 data, an aliquot of each of the initial samples (before heating) was taken for Absorbance measurement at $t_m > 16$ hours (the next day). In every case the results are identical within ± 2% (Note however, that the results for overnight samples are predominantly low (i.e., -2%), indicating that even long times do not drive the color development truly to "completion.")

As final evidence for the efficacy of the heat treatment modification, Figure 5 and Table IV present data for a real wastewater (WW) sample and (WW + SPK). WW (1:5) represents the quantitative outcome derived from a 1:5 dilution of the original sample before digestion; WW represents that from an equal aliquot of the same wastewater digested without prior dilution and subject to the heat treatment.

Beer's Law Plots for Various Development Times

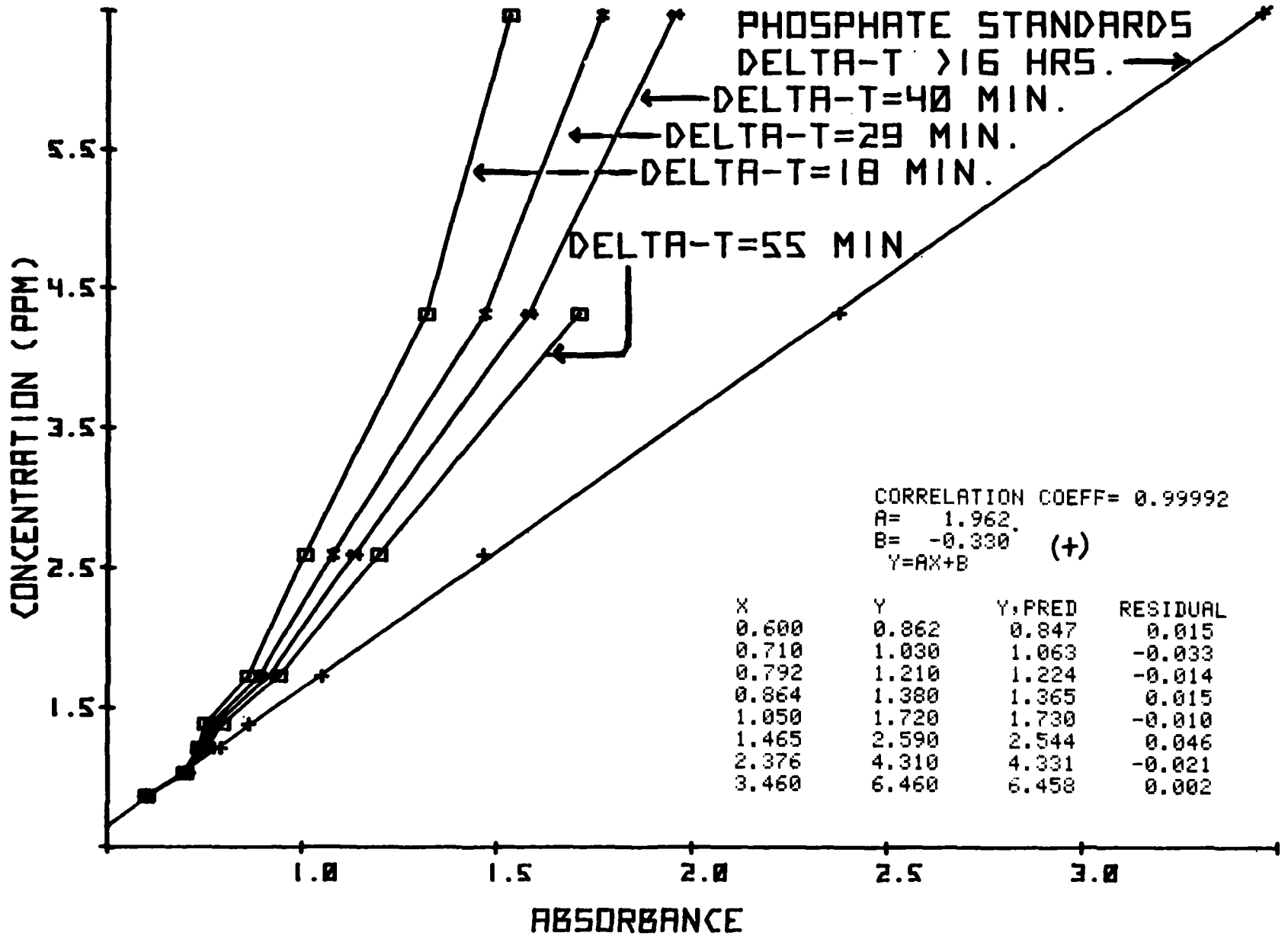


FIGURE 3

Beer's Law Plot after Development at 75 deg C for 20 Minutes

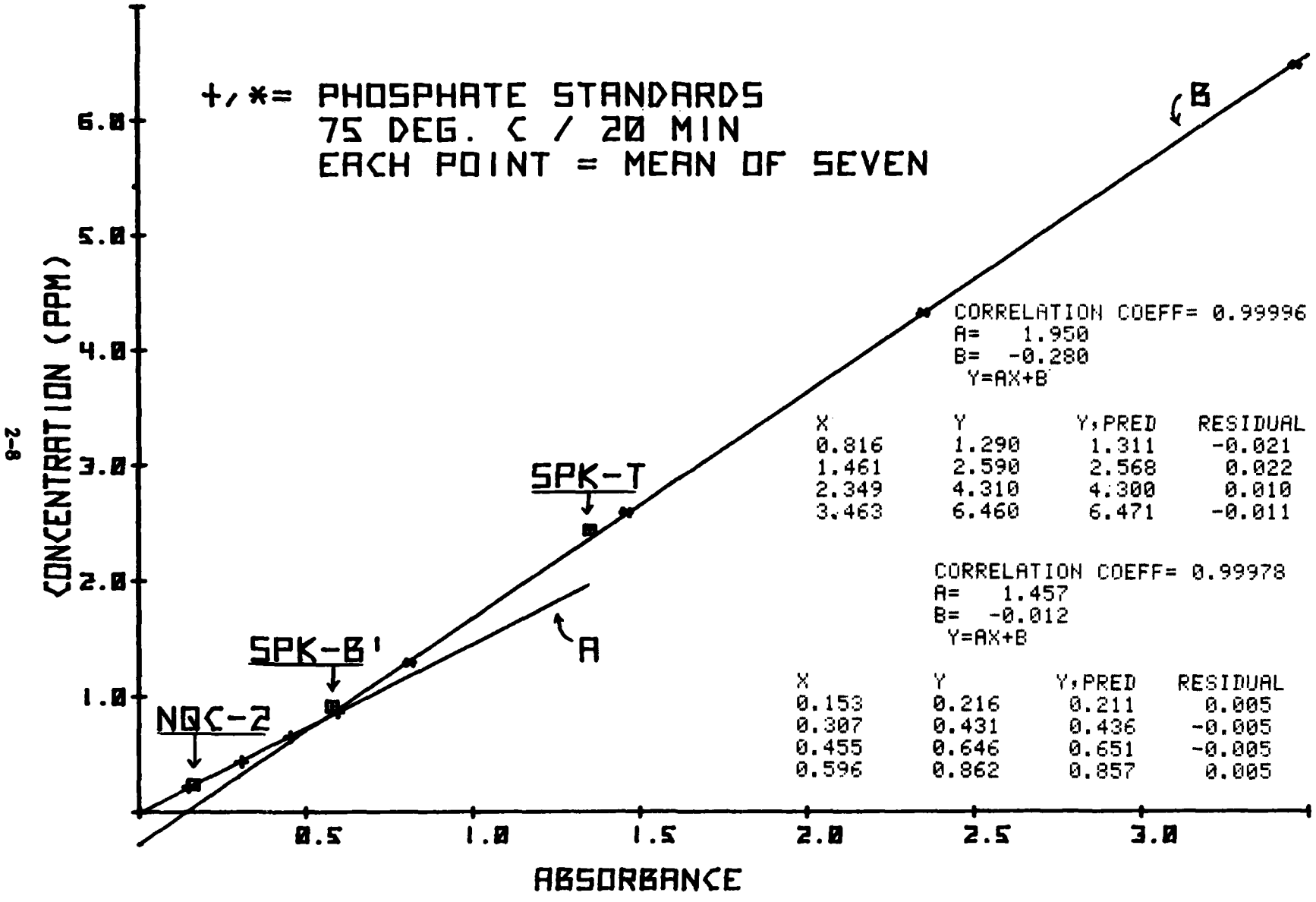


FIGURE 4

TABLE III

Statistical Data for Figure 4 Spikes

Replicate Number	NQC-2	SPIKE B'	SPIKE T
1	0.159	0.571	1.376
2	0.162	0.585	1.349
3	0.169	0.586	1.343
4	0.164	0.576	1.343
5	0.167	0.575	1.351
6	0.171	0.579	1.357
7	0.173	0.582	1.354
$\bar{A}$	0.166	0.579	1.353
S_A	0.005	0.006	0.011
RSD, %	3.0	1.0	0.8
		H * L	
$[PO_4^{3-}]^{ppm}_{obs}$	0.230		2.36
		0.849 0.832	
$[PO_4^{3-}]^{ppm}_{true}$	0.233	0.916	2.44
Recovery, %	98.7	92.7 90.8	96.6

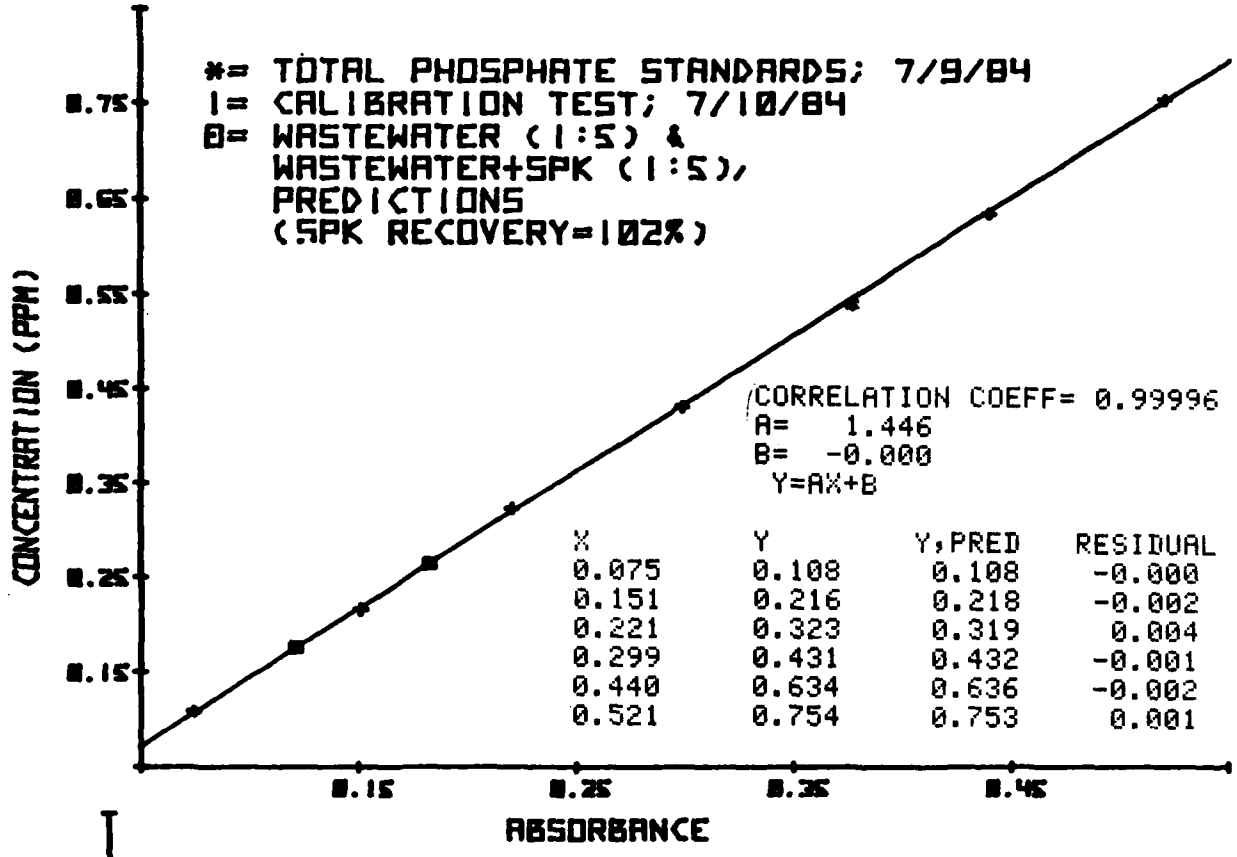
SPIKE B' = (20.0 mL · NQC-2) + 10.00 mL · (2.5 ppm).

SPIKE T = (20.0 mL · NQC-2) + 20.00 mL · (5.0 ppm).

*H = predicted from $[PO_4^{3-}]$ > 1.29 ppm.

L = predicted from $[PO_4^{3-}]$ < 0.862 ppm.

Beer's Law Plot of Standards and Wastewater Samples after Development at 75 deg C for 20 Minutes



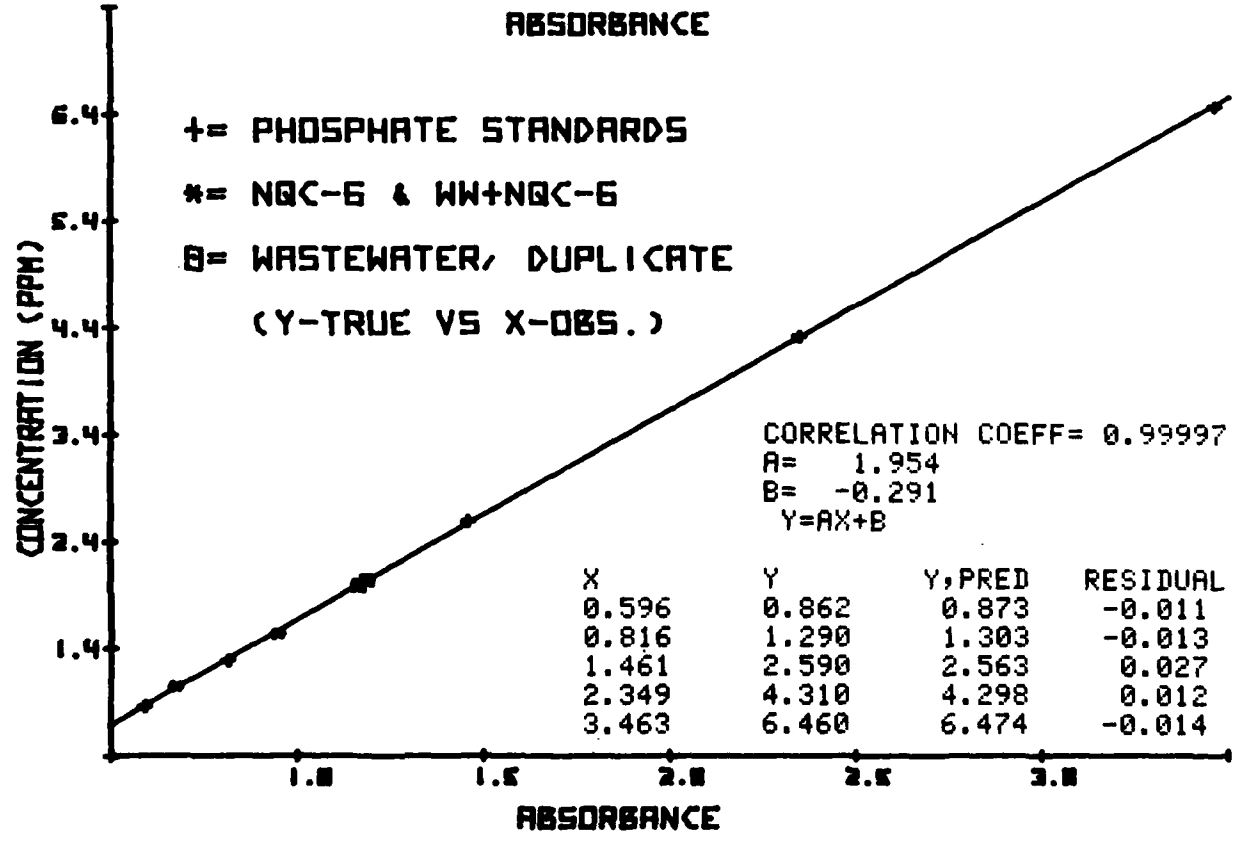


FIGURE 5

3.0 CONCLUSIONS

The single-solution ascorbic acid method for the determination of phosphorus as phosphate is the method of choice because of its sensitivity, and because of the relatively wide range in concentration amenable to 1.0 cm (standard) cuvettes.

The immediate use of disposable (polystyrene or acrylic) cuvettes represents no compromise in either the precision or the accuracy of quantitation (see below).

A procedure which includes the moderate, short-term heating of the developing sample enhances quantitative quality and eliminates the need for synchronizing the time of measurement either for samples in a given program or for calibration curves. Heating at 75 deg C for 20 minutes is an effective specification; one trial on Figure 4 samples at ca. 95 deg C for 30 minutes gave nearly identical results; there are indications that shorter time at 75 deg C or the same time at less than 75 deg C may also give equivalent results.

Fully developed samples which are not to be measured immediately can remain overnight in acid rinsed, glass containers (with secure covers) for "next day" measurement when necessary. Samples must not be left for extended times in plastic cuvettes.

Analysis at $[PO_4^{3-}] < 10$ ppm is insensitive to the amount of color developer (CD) used; however, for the purpose of ascertaining the inherent dilution factor, the exact aliquot of CD delivered must be noted.

Careful use of the graduated cylinder for dispensing purposes is sufficient.

The persulfate digestion process provides adequate recovery for total phosphate determination in the wastewater prevailing at RIA.

4.0 RECOMMENDATIONS

The single-solution ascorbic acid method with the modification presented here should be implemented for phosphorus (as phosphate) determinations.

Glassware should be allocated and sequestered for phosphate analysis only.

5.0 APPENDIX

5.1 Explication of Beer-Lambert Law.

The Beer-Lambert, sometimes called Bouguer-Beer Law, often referred to simply as Beer's Law, takes its name from the fact that it is phenomenologically composed from the application of two independent laws governing the absorption of radiant energy by colored solutions. One part, from the independent works of Lambert and Bouguer, concerns the change in transmitted intensity or power as a function of the "thickness" (pathlength) of solution traversed by the light; the second part, from the work of Beer, concerns the effect of number density of absorbers (concentration) on transmitted intensity or power through a fixed pathlength. Both laws are in turn based on the assumption of monochromatic radiation. This is a significant assumption and requires the bandwidth of radiation to be infinitely narrow for the rigorous validity of the Laws. Instrumentally this assumption can be met by assuring that the bandwidth of radiation employed is narrow compared to the width of the analytical absorption band used for quantitation (i.e., that $(dA/d\lambda) \simeq 0$ over the bandwidth of the wavelength being monitored.)

The Lambert Law can be expressed in exponential form as

$$P = P_o X^b \tag{1}$$

where P_o = incident power,
b = pathlength traversed by the radiant energy,
X = fraction of energy absorbed in transit per unit pathlength at unit concentration,
P = net transmitted power.

Similarly, the Beer Law, in exponential form, is

$$P = P_o X^c \tag{2}$$

where c = concentration of the absorber, and the other symbols have their previous meaning.

Both absorption Laws are examples of geometric series, and can be expressed as

$$\text{(Lambert)}\quad P = P_o \exp(-\kappa b) \tag{3a}$$

$$\text{(Beer)}\quad P = P_o \exp(-\mu c) \tag{3b}$$

κ and μ are proportionality factors determined by conditions of the experiment.

When combined to account for both effects,

$$P = P_o \exp(-abc) \tag{4}$$

where $a = \kappa\mu$ [in units of $(\text{pathlength} \cdot \text{concentration})^{-1}$]

In the more familiar logarithmic form, equation (4) is

$$\text{Absorbance, } A = \text{Log } (\frac{P_o}{P}) = abc$$

expressing what is known simply as "Beer's Law," and the constant a = absorptivity.

The point of this appendix is to emphasize that the porportionality of data obtained under different conditions of pathlength is rigorously contingent on the constancy of κ (equation 3a). As Figure 3 and its discussion show, κ is effectively not constant since the "unit" concentration per unit pathlength is still varying. Therefore in the range, $[PO_4^{3-}] > 1$ ppm, "Beer's Law" has not truly "broken down," rather the proportionality constant, a equation (4), has taken on a different value because of an intrinsic change in both κ and μ. A detailed analysis of the nature of this change is the subject of a forthcoming publication [6]. However, the results of this study strongly suggests that the changes in the intrinsic values of κ and μ are themselves linearly related so that an operational calibration curve may be constructed for the range above 1 ppm.

5.2 Spike (SPK) True Concentration and Recovery Calculations.

NB concentrations implied (=) by measured Absorbance are in terms of V_{TOT} = sample aliquot + CD aliquot.

SPK-A: 50.0 mL · (NOC-2) + 20.0 mL · (1.00 ppm std.)
V_{CD} = 8.00 mL
SPK-A = 0.429 ppm

SPK-B: 50.0 mL · (NOC-2) + 20.0 mL · (2.50 ppm std.)
V_{CD} = 8.00 mL
SPK-B = 0.814 ppm

SPK-C: 20.0 mL · (NOC-2) + 10.00 mL · (2.5 ppm std.)
V_{CD} = 3.20 mL
SPK-C = 0.916 ppm

SPK-D, SPK-DD: 20.0 mL · (NOC-2) + 20.00 mL · (2.50 ppm std.)
V_{CD} = 8.00 mL
SPK-D, SPK-DD = 1.282 ppm

SPK-B': 20.0 mL · (NOC-2) + 10.00 mL · (2.5 ppm std.)
V_{CD} = 3.20 mL
SPK-B' = 0.916 ppm

SPK-T: 20.0 mL · (NOC-2) + 20.00 mL · (5.0 ppm std.)
V_{CD} = 3.20 mL
SPK-T = 2.44 ppm

A. Wastewater Recovery (no dilution).

(1) WW = 50.0 mL wastewater

(a) one digested and brought to 50.0 mL, duplicate digested and brought to 60.0 mL;

(b) 20.0 mL aliquot + 3.20 mL CD gave:

A = 1.052 = $[PO_4^{3-}]$ = 1.76 ppm = 40.9 µg/20.0 mL (WW) = 102.4 µg

Per 50.0 mL original WW

A = 0.881 = $[PO_4^{3-}]$ = 1.43 ppm = 33.2 µg/20.0 mL (WW) = 99.6 µg

(2) WW + SPK = 50.0 mL · (WW) + 50.0 mL · (NQC-6)

(a) Two digested and brought to 50.0 mL;

(b) duplicate (20.0 mL + 3.20 mL CD) aliquots gave:

A = 1.554 = $[PO_4^{3-}]$ = 2.75 ppm = 63.7 µg/20.0 mL (WW + SPK) = 159 µg

Per 50.00 mL (WW + SPK)

A = 1.513 = $[PO_4^{3-}]$ = 2.66 ppm = 61.8 µg/20.0 mL (WW + SPK) = 154 µg

Per 50.0 mL (WW + SPK)

(3) (WW + SPK) mean recovery = 156 µg

- (WW) mean recovery = 101 µg

= (SPK) mean recovery = 55 µg

SPK added = (50.0 mL) · (1.04 ppm) = 52 µg

SPK % recovery = 55 µg/52 µg = 106%

Therefore, WW = 2.02 ppm without prior dilution

B. Spiked Wastewater Recovery (1:5 dilution).

(1) WW = (50.0 mL × (WW)/250.0 mL)

(a) Five 50.0 mL aliquots digested and each brought to 100.0 mL (NB, digestion procedure gives additional 2x dilution).

(b) 50.0 mL aliquot + 8.00 mL CD gave:

A = 0.122 = [PO_4^{3-}] = 0.176 ppm = 10.2 µg/50.0 mL [WW (1:10)]

or

A = 0.122 = [PO_4^{3-}] = 0.176 ppm = 20.4 µg/50 mL (WW)

(2) WW + SPK = [50.0 mL • (WW) + 20.0 mL • (2.5 ppm std.)] / 250.0 mL

(a) Five 50.0 mL aliquots digested and each brought to 100.0 mL (NB, digestion procedure gives additional 2x dilution).

(b) 50.0 mL aliquot + 8.00 mL CD gave:

A = 0.183 = [PO_4^{3-}] = 0.264 ppm = 15.3 µg/50.0 mL [WW + SPK (1:10)]

or

A = 0.183 = [PO_4^{3-}] = 0.264 ppm = 30.6 µg/50.0 mL (WW + SPK)

(3) WW + SPK mean recovery = 156 µg

- WW mean recovery = 101 µg

= SPK mean recovery = 55 µg

SPK % recovery = 10.2 µg/10.0 µg = 102%

Therefore, WW = (20.4 µg/50.0 mL) • 5 = 2.04 ppm

Compare 5.2.A.(3), WW = 2.02 ppm.

5.3 "Standard Method" Reagent Details.

(a) H_2SO_4 - 5N
70 mL conc. H_2SO_4/500 mL graduated cylinder

(b) $K(SbO)C_4H_4O_6 \cdot \frac{1}{2} H_2O$
1.3715g/500.0 mL (≅8.21 mM) volumetric flask

(c) $(NH_4)_6 Mo_7 O_{24} \quad 4 H_2O$
20g/500 mL (≅ 32.4 mM) graduated cylinder

(d) Ascorbic Acid (L-)
1.76g/1 mL (≅ 0.01 mM) volumetric flask

Combine in order with/mixing after each addition,

50.0 mL (a)
5.0 mL (b)
15.0 mL (c)
10.0 mL (d)

Stock (50 ppm) Phosphate

220.7 mg/L = 50.22 ppm ≅ 50 ppm.

6.0 REFERENCES.

1 Standard Methods for the Examination of Water and Wastewater, 15th Ed.; American Public Health Association; Washington, D.C.; 1980; section 424.

2 "Dilute" was interpreted as 10% v/v HCl; since a beaker of this acid rinse was routinely kept ready for use on a hot plate, the average actual concentration is probably much closer to 50% v/v.

3 Murphy, J. and Riley, J.; Anal. Chim. Acta, 27 (1962), p. 31-17.

4 All of the available methods involve a CD containing Mo (VI) as $(NH_4)_6Mo_7O_{24} \cdot 2H_2O$, ≅ 32mM.

5 US Environmental Protection Agency, 1979 "Methods for Chemical Analysis of Water and Wastes," EPA-600/4-79-020.

6 Towns, Theodore G., "A Critical Examination of the Single-Solution Ascorbic Acid Method," submitted to Analytical Chemistry.

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NOTES ON USE OF THE SINGLE SOLUTION ASCORBIC ACID METHOD FOR PHOSPHATE DETERMINATIONS: AN AMENDED METHOD

by Theodore G. Towns, Ph.D.

Report EN-84-15, December 1984, 30 p, incl. figures and tables. Unclassifed report.

The project objectives were to define the sources of potential inaccuracy and imprecision in ortho-phosphate analyses and to find a practical and reliable solution to quantitation on the interval 1 ppm $\leq [PO_4^{3-}] \leq$ 6 ppm with the use of 1.0 cm polystyrene cuvettes. The objectives were successfully completed. A modification to the current "standard method", involves heating samples, after addition of the color developer reagent, at 75 deg C for ca. 20 minutes. The modification yields quantitative results with high precision, high accuracy and virtually no constraint on the timing of absorbance measurements.

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2. Ascorbic Acid Method
3. Wastewater
4. Colorimetry
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